

CLAIMS

1. A method for preparing a F(ab')₂ fragment from a glycosylated antibody, said method comprising the steps of:

- (i) providing said glycosylated antibody, said glycosylated antibody having a hinge region having one or more protease cleavage sites located within said hinge region, one or more non-hinge regions adjacent said hinge region, said non-hinge region(s) having one or more oligosaccharide groups attached thereto, said oligosaccharide group(s) causing said protease cleavage site(s) within said hinge region to be resistant to a proteolysis treatment;
- (ii) exposing said glycosylated antibody to a deglycosylation treatment, said deglycosylation treatment cleaving said oligosaccharide group(s) attached to said non-hinge region(s) to form a partially or wholly deglycosylated antibody having a hinge region cleavable by said proteolysis treatment; and,
- (iii) exposing said partially or wholly deglycosylated antibody to said proteolysis treatment to cause proteolytic cleavage of said hinge region cleavable by said proteolysis to form said F(ab')₂ fragment.

2. The method of claim 1 wherein said glycosylated antibody is a plurality of glycosylated antibodies.

3. The method of claim 2 wherein at least some of said glycosylated antibodies are polyclonal.

4. The method of claim 2 wherein said glycosylated antibodies are monoclonal.

5. The method of claim 1 wherein said glycosylated antibody is either an IgG₁ or IgG_{2b} glycosylated antibody.

6. The method of claim 5 wherein said IgG₁ or IgG_{2b} antibody is from a rodent derived hybridoma cell culture or ascites.
7. The method of claim 1 wherein said glycosylated antibody is derived from the group consisting of rat, mouse, rabbit, goat, sheep, lamb, chicken, or horse.
8. The method of claim 1 wherein said proteolysis is achieved wholly or partly from protease treatments including components selected from the list consisting of pepsin, proteases that cleave pepsin substrates, papain, papain preactivated with cysteine, and ficin.
9. The method of claim 1 wherein said proteolysis is achieved by a protease capable of producing F(ab')₂ fragments from said deglycosylated antibodies.
10. The method of claim 1 wherein said deglycosylase treatment contains a glycosidase combination selected from the group consisting of PNGase F, endo-O-glycosylase, sialidase A, PNGase F/endo-O-glycosylase, PNGase F/ sialidase A, PNGase F/endo-O-glycosylase/sialidase A, endo-O-glycosylase/sialidase A.
11. The method of claim 1 wherein said non-hinge regions comprise the Fc and the Fab regions of said glycosylated antibody.
12. A method for preparing F(ab')₂ fragments comprising the steps of:
 - (i) growing a hybridoma cell that normally produces glycosylated antibodies having a hinge region with one or more protease cleavage sites located within said hinge region, one or more non-hinge regions adjacent said hinge region, and one or more oligosaccharide groups being attached to at least one of said non-hinge regions by said hybridoma cell through glycosylation, said oligosaccharide groups causing said hinge regions to be resistant to a proteolysis treatment;

- (ii) administering to said hybridoma cell an inhibitor of said glycosylation effective to inhibit glycosylation of said antibodies to produce one or more unglycosylated antibodies lacking said oligosaccharides within at least one non-hinge region to cause said hinge region to be prone to said proteolysis treatment; and,
 - (iii) exposing said unglycosylated antibodies to said proteolysis treatment, wherein said unglycosylated antibodies' hinge regions are cleaved to form said $F(ab')_2$ fragments from said unglycosylated antibodies.
13. The method of claim 12 wherein said hybridoma cell is part of a hybridoma cell culture or ascites.
14. The method of claim 12 wherein said hybridoma cell is a plurality of hybridoma cells.
15. The method of claim 12 wherein said hybridoma cell is part of a monoclonal or polyclonal hybridoma cell line.
16. The method of claim 14 wherein said hybridoma cells are from the same hybridoma cell line.
17. The method of claim 14 wherein said hybridoma cells are from different hybridoma cell lines.
18. The method of claim 12 wherein said inhibitor of said glycosylation contains bacitracin or tunicamycin.
19. A method for preparing $F(ab')_2$ fragments comprising the steps of:
- (i) providing a hybridoma cell line that normally produces glycosylated antibodies having a hinge region with one or more protease cleavage sites located within said hinge region, one or more non-hinge regions adjacent said

hinge region, and one or more oligosaccharide groups being attached to at least one of said non-hinge regions by said hybridoma cell through glycosylation, said oligosaccharide groups causing said hinge regions to be resistant to a proteolysis treatment;

- (ii) altering said hybridoma cell line to inhibit glycosylation of said antibodies within said non-hinge regions to produce one or more unglycosylated antibodies such that said unglycosylated antibodies are susceptible to proteolysis treatment;
- (iii) causing said hybridoma cell line to produce said unglycosylated antibodies; and,
- (iv) exposing said unglycosylated antibodies to said proteolysis treatment to cleave said unglycosylated antibodies to produce said F(ab')₂ fragments.

20. The method of claim 19 wherein said hybridoma cell is part of a hybridoma cell culture or ascites.

21. The method of claim 19 wherein said altered cells are either permanently or transiently altered.

22. An F(ab')₂ composition comprising:

one or more F(ab')₂ fragments, or derivative therefrom, produced by the method selected from the group consisting of the methods of claim 1, claim 12, and claim 19.

23. The composition of claim 22 wherein said F(ab')₂ fragments are an active ingredient of an antitoxin or anti-venom medicament.

24. An immunoglobulin composition comprising:

one or more aglycosylated or deglycosylated immunoglobulins, said aglycosylated or deglycosylated immunoglobulins being formed by preventing the attachment of one or more oligosaccharides to said immunoglobulin, or effecting the removal of an attached

oligosaccharide from said immunoglobulin by exposure to one or more deglycosylases, or both by preventing attachment to and removing one or more oligosaccharides from said immunoglobulin, wherein at least one of said one or more aglycosylated or deglycosylated immunoglobulins becomes cleavable by a protease which cleaves said aglycosylated or deglycosylated immunoglobulins at a position to form F(ab')₂ fragment(s) from said aglycosylated or deglycosylated immunoglobulins as a result of said immunoglobulin being aglycosylated or deglycosylated.

25. A kit for making F(ab')₂ fragments from one or more immunoglobulins, at least one of said immunoglobulins having one or more oligosaccharides attached thereto that inhibit protease activity that converts said immunoglobulins into F(ab')₂ fragments comprising: a deglycosylation composition containing one or more deglycosylase enzymes or chemicals capable of removing or reducing some or all of said oligosaccharides; and, a protease composition containing one or more proteases capable of reacting with said immunoglobulin produces F(ab')₂ fragments from said deglycosylated antibodies.

26. The kit of claim 25, further comprising a purification medium for purifying said F(ab')₂ fragments from non-F(ab')₂ fragments of said immunoglobulin or from uncleaved immunoglobulin.

27. The kit of claim 25, further comprising instructions for carrying out the method selected from the group consisting of the methods of claim 1, claim 12, or claim 19.